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Published in:
Metabolism

DOI:
10.1016/j.metabol.2019.04.014

Publication date:
2019

Document version
Publisher's PDF, also known as Version of record

Metabolism Clinical and Experimental 96 (2019) 46–55

Contents lists available at ScienceDirect

Metabolism Clinical and Experimental

journal homepage: www.metabolismjournal.com

Article from the Incretin Hormones and Incretin-based Glucose-lowering Medications Special Issue, Edited by Michael Nauck, Manfredi Rizzo and Christos Mantzoros

The incretin system in healthy humans: The role of GIP and GLP-1

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ARTICLE INFO

Article history:
Received 18 March 2019
Received in revised form 11 April 2019
Accepted 23 April 2019

ABSTRACT

The incretin effect, the amplification of insulin secretion occurring when glucose is taken in orally as compared to infused intravenously, is one of the factors that help the body to tolerate carbohydrate/glucose ingestion. These include 1) amount and type of carbohydrates; 2) gastric emptying rate; 3) digestion and absorption of the carbohydrates; 4) secretion and effect of the incretin hormones; 5) disposition of absorbed nutrients/glucose. The incretin effect can also be viewed as the fraction of the ingested glucose load handled via gastrointestinal mechanisms (including the incretin effect); it is calculated by comparison of the amount of glucose required to copy, by intravenous infusion, the oral load. Typically, for 75 g of oral glucose, about 25 g are required. This means that the GastroIntestinal Glucose Disposal (GIGD) is 66%. Both the GIGD and the incretin effect depend on the amount of glucose ingested: for higher doses the GIGD may amount to 80%, which shows that this effect is a major contributor to glucose tolerance. The main mechanism behind it is stimulation of insulin secretion by a proportional secretion of the insulinotropic hormones GIP and GLP-1. Recently it has become possible to estimate their contributions in healthy humans using specific and potent receptor antagonists. Both hormones act to improve glucose tolerance (i.e. the antagonists impair tolerance) and their effects are additive. GIP seems to be quantitatively the most important, particularly regarding insulin secretion, whereas the action of GLP-1 is mainly displayed via inhibition of glucagon secretion.

1. Introduction - Factors Regulating Glucose Tolerance

Strictly speaking, the incretin effect refers to the amplification of insulin secretion, which occurs when glucose is taken in orally as compared to infused intravenously [1]. Normally, the insulin response is augmented by a factor of 2–3 after oral intake. Physiologically, this phenomenon is one of the ways whereby the organism copes with the intake of a carbohydrate load. Thus, it is one of the important mechanisms governing *glucose tolerance*. Glucose tolerance is usually defined as the plasma glucose profile after intake of a certain amount of carbohydrate (glucose) relative to what is seen in a group of unquestionably healthy individuals given the same carbohydrate meal. Impaired glucose tolerance therefore refers to concentrations exceeding normal boundaries of the excursions. Until recently, these boundaries were essential for a diagnosis of impaired glucose tolerance or type 2 diabetes, whereas today emphasis is on the integrated glucose levels, as reflected in the concentration of glycated haemoglobin, haemoglobin A1c. So how is glucose tolerance normally regulated? Looking at impairments in glucose metabolism, it is customary to analyse fasting glucose concentrations and postprandial glucose excursions separately, and this is reasonable since the mechanisms involved are different [2]. Of course, if fasting glucose concentrations are elevated, the postprandial glucose profile, everything else being equal, will also be shifted upwards, so that postprandial levels are also abnormal, while at the same time the gastrointestinal mechanisms for handling oral carbohydrate loads may be completely intact. An example could be patients with steatosis of the liver, where hepatic insulin resistance and hyperglucagonemia may explain fasting hyperglycemia, but have limited impact on postprandial events. Also in patients with type 2 diabetes, it is reasonable to distinguish between the postprandial and the fasting glucose levels, because the two are independently associated with cardiovascular risk [3]; thus, in subjects with well-controlled diabetes, postprandial glycemia contributes relatively more to HbA1c than fasting hyperglycemia, whereas fasting glucose contributes relatively more in subjects with dysregulated type 2 diabetes [4]. The incretin effect, the topic of the present review, is obviously one of the important determinants of the postprandial glucose excursions.

Systematically, the following factors are important for postprandial glucose excursions [5]:

1. The load of carbohydrates

This seems self-evident, but is nevertheless an important factor in the planning of diets for people with glucose intolerance. Only recently has it become generally accepted that low carbohydrate diets have a major beneficial effect on the course and risk of complications in

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https://doi.org/10.1016/j.metabol.2019.04.014

patients with T2DM, with even moderate reductions resulting in significantly improved haemoglobin A1c levels and reduced liver fat (and thereby improved insulin sensitivity and increased insulin clearance) [6,7]. A related issue is the glycemic index of the ingested carbohydrates – the lower the glycemic index, the lower the postprandial glucose levels, and again it is assumed that such reductions will reduce the cardiovascular risk [7].

2. Gastric emptying rate

Once the meal is ingested, the next important factor is the gastric emptying rate. Clearly, liquid meals are emptied at faster rates than solid meals and with different kinetic patters (exponential patterns for liquids and, after a lag phase, linear for solids), but on top of that the emptying rate is strictly regulated by the meal composition to result in a rather constant transfer of nutrients to small intestine, corresponding to between 1 and 4 kcal/min [8]. The actual emptying process consists of a brief opening of the pyloric sphincter associated with a strong propulsive contraction of the antrum, resulting in a rapid ejection a bolus of gastric contents into the duodenum, where peristalsis activated by the possibly acidic bolus secures further onward transportation to more distal segments of the jejunum. In this way a rather large mucosal surface of the upper small intestine is rapidly exposed to the nutrients. Effective upper intestinal mechanisms are now activated which result in a feedback, involving both long vago-vagal reflexes, probably also short, intramural reflexes, as well as endocrine mechanisms (cholecystokinin, secretin, perhaps also GLP-1, somatostatin?), secreted in response to both acidity, osmolarity and specific nutritional constituents (glucose, proteins, lipids), which all powerfully dampen further emptying from the stomach [5,9]. The result is an adjustment of nutrient transfer to the small intestine that depends on the composition and caloric density of the chyme delivered to the small intestine. In this way the emptying of an energy-dense meal will be spread out in time, presumably with the purpose of preventing untoward effects of rapid emptying (early and late dumping) and excessive increases in postprandial nutrient (e.g. glucose) concentrations in plasma.

3. Digestion and absorption

In the small intestine, digestion and absorption of the nutrients are the next processes with an impact on the resulting plasma profile. Normally, neither carbohydrate digestion nor absorption are limiting factors, which is illustrated by the rapid and extensive absorption of glucose in conditions with accelerated gastric emptying (e. g. operations where pyloric function has been distorted) where rapid increases to abnormally high plasma levels may be seen [10]. For complex carbohydrates, the story is of course more complicated. However, the absorption rate is of great importance for the next-in-line regulator of glucose tolerance, the incretin effect. This is because the secretion of the incretin hormones is dependent on the absorption rate of the nutrients (glucose/amino acids) by the endocrine cells responsible for their secretion [11,12].

4. The incretin effect

The incretin effects now sets in with its actions on the endocrine pancreas, and in particular insulin secretion [13]. The details of this will be discussed below.

5. Disposition/deposition of nutrients

The next and final, important step in glucose tolerance is the disposition of the nutrients/glucose. The most important factor is of course insulin, which switches the liver from a site of glucose production in the fasting state to a site where part of the absorbed glucose may be taken up and stored as glycogen. Changes in glucagon secretion are also important for this change in the liver's metabolic function, with glucagon secretion normally being inhibited by the increasing plasma levels of glucose and some of the gastrointestinal hormones, in particular GLP-1. Impaired braking of hepatic glucose production (which is already elevated in T2DM) will clearly have untoward effects on glucose tolerance as discussed above. Most of the absorbed glucose, however, travels further on to the peripheral tissues, where two mechanisms are particularly important for the rate of glucose disposal: the mass action of the elevated glucose concentration (also sometimes referred to as glucose effectiveness) [14,15] and insulin's effect on glucose uptake in muscles and adipose tissue. Given the presence of glucose transporters in the tissues, it is clear that increases in the plasma concentration of glucose results in increased transfer of glucose to the intracellular compartment by facilitated diffusion. Insulin's effect on glucose transport involving the insertions of additional glucose transporters (GLUT4) in the plasma membranes of the fat and muscle cells will augment the diffusion even further. The postabsorption details and a discussion of the mechanisms of action of insulin receptor signaling are beyond the scope of this presentation, but clearly the intracellular fate of glucose may affect the rate of transfer, being dictated by the gradients driving the diffusion; thus removal of glucose from the relevant membrane areas (by phosphorylation) is essential for continued transport and, similarly, further metabolism of glucose-6-phosphate is important to prevent substrate inhibition of this process [16]. In the glucose tolerant individual, the final deposition of glucose comprises oxidation and/or storage as glycogen. These processes can be greatly accelerated in healthy individuals (suggesting surplus capacity) [17], but may be severely compromised in T2DM and may therefore influence postprandial glucose levels.

2. The Incretin Effect

2.1. Definition and Calculation. Gastrointestinally Mediated Glucose Disposal (GIGD)

Among all these factors, how important is the incretin effect? It is easy to quantitate the effect in terms of insulin responses: this is simply done by comparing the insulin responses (the areas under the curve of the insulin responses, or the C-peptide responses, whereby differences in hepatic extraction of insulin are avoided; or ultimately, insulin secretion rates calculated by de-convolution of C-peptide responses) to an oral glucose load and an intravenous load adjusted so as to give rise to the same peripheral (arterial) glucose concentrations (isoglycemia) [18]. In this situation, the beta cells experience the same arterial glucose concentrations and the difference between the oral and the intravenous response is due to these gastrointestinal factors, the incretin effect. From this comparison, it can be calculated that the incretin affect may be responsible for up to 70% of the insulin response to oral glucose. But can the incretin effect be converted into its importance for glucose tolerance? Does it follow that an impaired incretin response will be associated with a similarly impaired glucose tolerance? [19].This important question has not until recently (see below) been properly addressed. An alternative way of looking at the incretin effect is to measure the amount of intravenous glucose required to copy the oral curve. For instance, how much glucose does it take to copy, by infusion, the glucose response to a 75 g oral load? It turns out to be around 20–25 g in young healthy individuals. The difference is about 50 g. In other words, the oral intake involves activation of mechanisms that enable the body to dispose of 2/3 of the ingested load in addition to those activated by the increases in plasma glucose alone [18]. This amount has sometimes been referred to as GIGD, gastrointestinally mediated glucose disposal [20,21], and GIGD amounts to 66% in this example. GIGD has by some been called “the poor man's incretin estimate”, but actually the GIGD is the physiologically relevant parameter, addressing the question: how good is the body to mitigate the challenge of a large carbohydrate

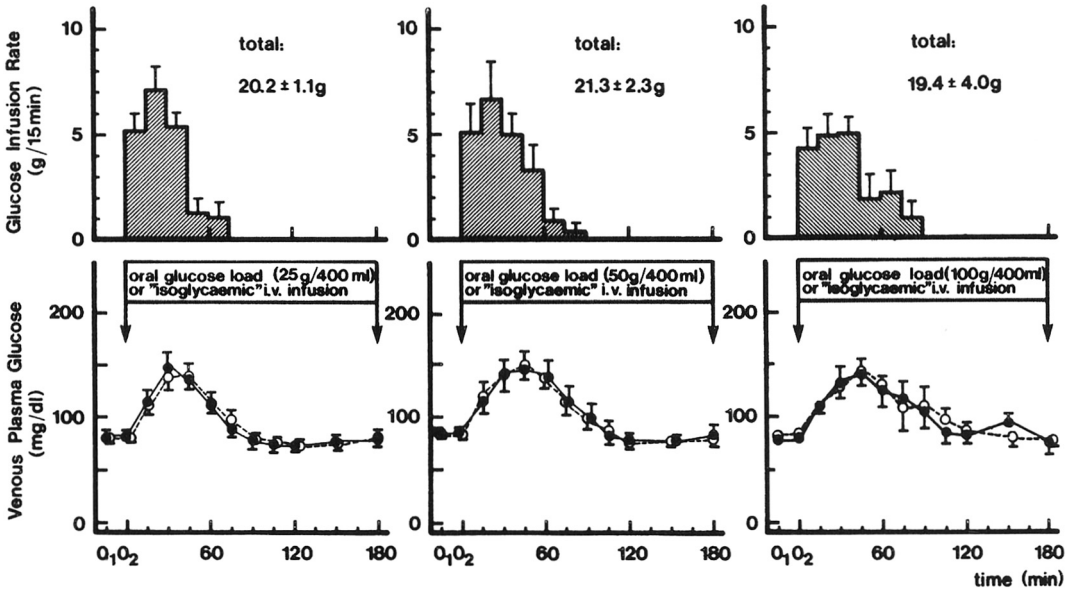

Fig. 1. Plasma glucose responses in 6 young healthy subjects to ingestion of increasing amounts of glucose (25 g, 50 g 75 g) and intravenous infusions of glucose designed to copy the plasma profiles after oral ingestion. The average amount of glucose required for the infusions is indicated in the top panel. Men +/− SEM. From Nauck et al. [18]with permission.

meal? Whether this is due to insulin release, glucagon inhibition, liver and tissue uptake, etc. are details that we can deal with once we have got an impression of how well the body handles the carbohydrate load. In fact, GIGD can be calculated also in type 1 diabetic subjects (where the incretin effect of course has no meaning), in whom it may actually reach negative values! [22].

2.2. The Incretin Effect is Dose-dependent

So, is the incretin effect a constant figure? The amount of carbohydrate we ingest is highly variable, so perhaps it is not surprising that the incretin effect is also variable. As mentioned, the effect may be estimated from the amounts of intravenous glucose required to copy the oral administration. In elegant experiments, Nauck et al. [18] looked at the incretin effect after ingestion of 25, 50 and 100 g of glucose (in equal volumes) (Fig. 1). The immediately most striking result was that virtually identical plasma glucose concentrations resulted from these ingestions, regardless of the dose administered. One can conclude that the body has a very effective mechanism that keeps the plasma glucose concentrations low regardless of the amount of glucose we ingest. So how much glucose was infused to copy these curves? About 20 g were required for the 25 g dose and, as expected, about 20 g were required after the other two oral doses as well. So the GIGD mechanism is sufficient to account for as much as 80% of the disposal of an oral glucose load of 100 g! But what is the explanation? The explanation is apparent by inspection of the insulin curves (Fig. 2), which show a progressive and massive increase in insulin secretion elicited in spite of the identical plasma glucose concentrations [18].

2.3. The Incretin Hormones

The question then arises, which factors are responsible for this excess stimulation. Here, the so-called incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are probably the most important [13]. These peptides of the glucagon – secretin family of peptides are secreted from

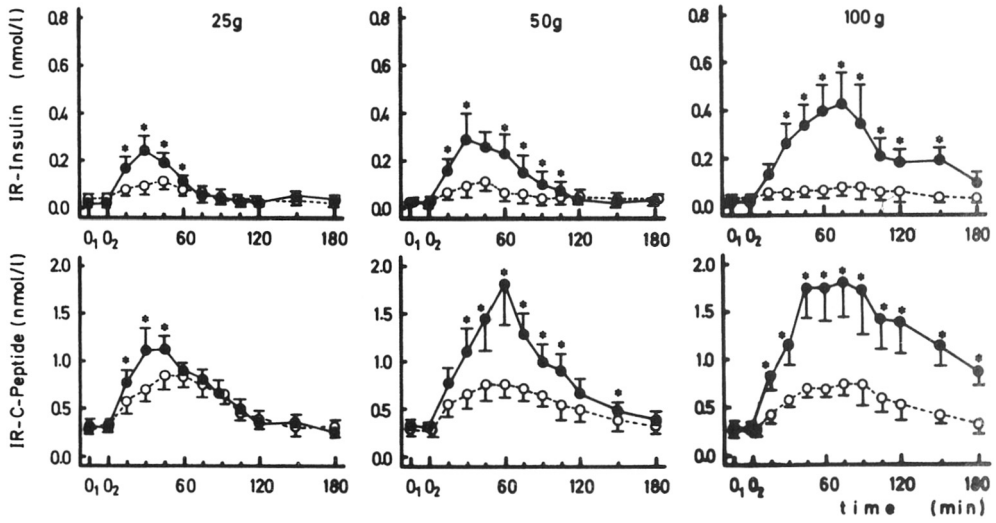

Fig. 2. Insulin and C-peptide responses from the experiments show in Fig. 1. Filled circles: oral glucose ingestion; open circles: intravenous glucose infusion. Mean +/− SEM. Asterisks indicated significant differences. From Nauck et al. [18] with permission.

Table 1
Summary of the effects of GLP-1 and GIP: similarities and differences.

Effects	GIP	GLP-1
Beta cell proliferation/apoptosis inhibition	↑↑	↑↑
Insulin secretion	↑↑↑	↑↑↑
Insulin secretion in T2DM	(↑)	↑↑
Glucagon secretion	↑↑	↓↓↓
Somatostatin (pancreatic) secretion	↑↑	↑↑
appetite	→	↓↓
Food intake	→	↓↓
Body weight	→	↓↓
Efferent vagal activity	→	↓↓
Gastric emptying	→	↓↓[a]
Gastro-pancreatic secretion	→	↓↓[a]
Mesenteric blood flow	↑	→
Adipose tissue blood flow	↑↑	→
Heart rate	↑	↑↑
Adipocytes (lipolysis, lipid uptake)[b]	↑↑	→
Bone resorption	↓↓↓	↓
Bone formation	↑	→

[a] Via inhibition of the efferent vagus.
[b] Lipolysis in the absence of insulin, net lipid uptake in the presence of insulin.

endocrine cells in the small intestine in response to nutrient ingestion; glucose is a particularly powerful stimulus for both, and the secretion of them also depends on the amount of glucose ingested (Table 1). In experiments with increasing oral doses of glucose, plasma concentrations of both GIP and GLP-1 were measured [23] (Fig. 3). It is clear that the secretion of both is stimulated very rapidly, probably at the first emptying of gastric contents into the small intestine, and then continues at a rate which is proportional with the graded emptying of the stomach contents. Thus, the duration of the response, not the amplitude, depends on the total amount of glucose ingested in agreement with the observation that the emptying rate is kept rather constant.

3. GIP and GLP-1

3.1. Secretion of GLP-1 and GIP

The incretin hormones are peptides of 42 and 30 amino acids, respectively, and are secreted by open type endocrine cells of the intestinal epithelium, the so-called K- and L-cells [24]. The density of the K-cells is very high in the duodenum and proximal jejunum, whereas the L–cells are more numerous more distally, and are even found at high densities in the colon [25]. This immediately suggests that they may have different roles, with GIP acting as an early incretin and GLP-1 perhaps playing a greater role later. Nevertheless, secretion of the two hormones generally shows up at the same, very early time point [26], and experiments in rats have shown that the upper half of the small intestine can produce GLP-1 with the same kinetics and in similar amounts as the distal small intestine [27]. However, in a recent human study [21] involving intestinal intubation, intraluminal infusions of glucose in either the mid duodenum or 177 cm further down showed more GIP and less GLP-1 upon duodenal infusion, and more GLP-1 and less GIP upon distal infusion, which would seem consistent with their anatomical distribution. The two cell types are of the open type, meaning that an apical process of the cells equipped with microvilli reaches the intestinal lumen [28,29]. The luminal membranes are thought to be equipped with SGLT-1 co-transporters allowing entry of glucose into the cells [30]. The simultaneous entry of sodium ions is thought to cause a depolarization of the membrane potential leading to opening of calcium channels, and exocytosis of the contents of hormone-containing granula from the basolateral side [30]. Thus, inhibition of these voltage-gated calcium channels with nifedipine blocks the effect of luminal glucose [31]. The important observation here is that secretion is coupled to absorption which explains the relationship between the glucose absorption profiles and hormone release [12].

3.2. Incretin Receptors and Signaling

The two incretin hormones have specific receptors (a single type for each) that are expressed in high numbers on the beta cells explaining that elevated plasma concentrations may result in stimulated insulin secretion [32,33]. Both receptors couple to a GαS protein mediating activation of adenylate cyclase and cAMP formation. Activation of protein kinase A as well as the EPAC2-pathway is responsible for further signaling leading to insulin granule exocytosis [34,35]. Both hormones also activate transcription of genes required for insulin production and for most other essential proteins in beta cell function, and both hormones may exert trophic actions on young beta cells, leading to proliferation [35,36]. The latter is of course of major clinical interest, but the response seems to limited to very young beta cells [37], very different from those of middle aged or older patients with type 2 diabetes, in whom a proliferating effect apparently has not been seem. Both hormones also seem to be able to inhibit beta cell apoptosis, induced by both cytokines and fatty acids [38], and with beta cell apoptosis apparently being a major problem in T2DM [39], this may be equally clinically important. In essence, both hormones potentiate glucose-induced insulin secretion, meaning that they do not have any effect in the absence of a glucose stimulus [40,41]. Although not completely worked out, it is possible that they interact with K_{ATP}-channel activity in a PKA dependent manner, facilitating inhibition of channel activity by ATP generated in glucose metabolism [35]. Whatever the mechanism, the result is that the peptides normally cannot cause hypoglycaemia, because they lose effectiveness as glucose concentrations fall [42]. In support of this theory, it has been found that the glucose dependency of GLP-1's effect on insulin secretion can be uncoupled by sulfonylurea compounds acting to inhibit the KATP channels [43]. This has clinical implications, by increasing the risk of hypoglycaemia when GLP-1 receptor agonists are combined with sulfonylureas. It has also been suggested [44] glutamate, derived from the malate-aspartate shuttle upon glucose stimulation, underlies the stimulatory effect of incretins and that glutamate uptake into insulin granules mediated by cAMP/PKA signaling amplifies insulin release. This is of particular interest since glutamate production is diminished in pancreatic islets of animal models of diabetes, while a membrane-permeable glutamate precursor restored amplification of insulin secretion in these models [44]. Thus, cytosolic glutamate may represent the elusive link between glucose metabolism and cAMP action in incretin-induced insulin secretion.

3.3. Comparing GIP and GP-1

So are the two hormones completely equivalent? GLP-1 seems to be more potent than GIP [45], but the major difference lies in the effectiveness of the two hormones in type 2 diabetes, where GLP-1 retains it stimulatory activity, whereas that of GIP is almost completely lost [46,47]. This phenomenon is currently unexplained. Down regulation of GIP receptor in T2DM has been proposed, supported by animals studies, where a down regulation was observed in experimental diabetes [48,49]. In humans, this does not seem to explain the difference; there *is* actually a small early insulin response to GIP during a hyperglycemic clamp in patients with T2DM (of little consequence for glucose turnover), whereas the late phase response of insulin secretion is completely lost [47]; in contrast, in the same individuals, GLP-1 may restore insulin secretion to the rates of non-diabetic controls, exposed to the same clamp (but without GLP-1). However, the early phase response to GLP-1 is also reduced in patients with T2DM, and if one compares the patients' early responses to GIP and GLP-1 with those obtained in healthy controls, their relative magnitude is exactly the same [47]. This must mean that the receptor expression is unchanged (or changed similarly for GIP and GLP-1), and that post receptor events, therefore, must explain the GIP failure.

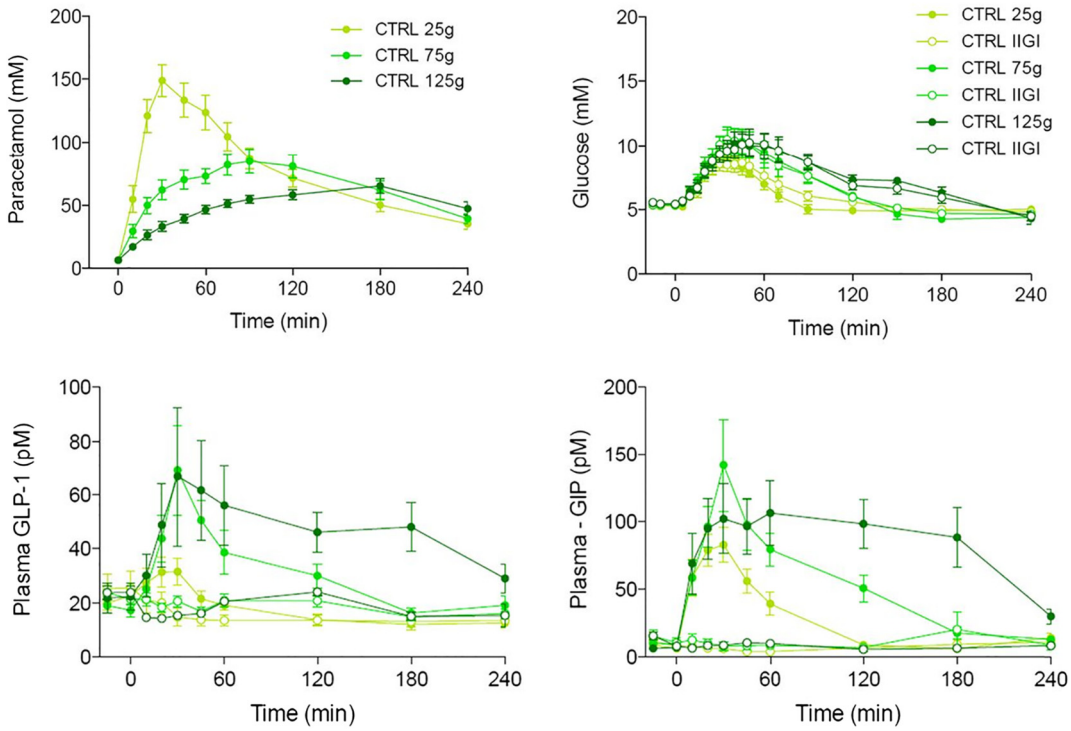

Fig. 3. Paracetamol, glucose, GLP-1 and GIP concentrations in plasma in 8 healthy volunteers after oral ingestion of 25, 75 and 125 g of glucose as well as intravenous glucose infusions designed to copy the profiles after oral ingestion. Mean +/− SEM. From Bagger et al. [23]with permission.

3.4. The Importance of Incretin Postsecretory Metabolism

The two hormones also exhibit another important difference. Fig. 2 shows the secretion profiles of the two hormones, but do these profiles correspond to their impact on the beta cell? This turns out not to be the case, and the difference lies in their postsecretory fate. Both are secreted in an intact, fully active form: GLP-1 as an amidated peptide of 30 amino acids [50], usually, for historical reasons, designated GLP-1 7–36 amide, GIP as a non-amidated 42-amino acid peptide. But both peptides are substrates for the almost ubiquitous enzyme, dipeptidyl peptidase 4, which is circulating, but also bound to cell membranes in the liver, the kidneys and to the luminal surface of endothelial cells [51]. The enzyme cleaves off the two N-terminal amino acids, leaving behind truncated peptides, which have lost their insulinotropic properties and actually may act as, rather weak, receptor antagonists [52,53]. The DPP-4 mediated degradation leaves GIP with a half-life in the circulation of 7 min [54], which can be determined with assays for the intact peptide, either C-terminal radioimmunoassays, or even better, sandwich ELISAs relying on antibodies against both of the intact termini of the molecule. The metabolite has a half-life around 30 min consistent with an elimination predominantly due to glomerular filtration in the kidneys. In humans, around half of circulating GIP is present in the intact form, and this must be considered when plasma profiles obtained with assays for total GIP (i. e. the intact hormone + the metabolite, which is what is measured with assays directed at the C-terminus) are evaluated [55]. For GLP-1, the degradation is even more extensive. GLP-1 is exquisitely sensitive to DPP-4 and most of the newly secreted GLP-1 is broken down already in the capillaries of the gut, so that only about 2/3 or 1/4 of what arrives to the liver remains intact [50]. In the liver, 50% of what is presented is broken down so that in total about 12% of what was secreted arrives to the systemic circulation in the intact form [56]. And, because of the soluble DPP-4, it has been found that only about 8% of what was released arrives at the peripheral targets (e. g. the endocrine pancreas) in the intact form [57]. The half-life observed in infusion studies is around 1–2 min, but the plasma clearance is up to 3 times the cardiac output [58], indicating that there is no equilibrium and that the peptide is continuously broken down at multiple sites in the circulation. So, how can the hormone be effective under these circumstances? After particularly large meals, there *is* a measurable increase in the concentration of the intact hormone, which is otherwise very low and most often undetectable, and this increase may of course influence beta cell function [59]. However, numerous observations support that GLP-1 mainly acts by activation of sensory vagal afferents expressing the GLP-1 receptor [60] (Fig. 4). There is a dense network of nerve fibres staining positively for the GLP-1 receptor in the intestinal wall [61], and many cell bodies in the nodose ganglion (harbouring the cell bodies of the vagal afferents) express GLP-1 receptor mRNA [62]. These sensory fibres terminate in the nucleus of the solitary tract (NTS) in the brain stem, and after peripheral administration of GLP-1, c-fos activation can be observed in a subset of nuclei in the NTS [63]. Efferent vagal motor nuclei in the dorsal vagal complex may also show signs of activation, suggesting that the efferent vagus is also stimulated [64]. In other words, long vago-vagal reflexes may transmit the signals generated by peripheral GLP-1, acting on nerve fibres in the gut wall and before it is taken up by capillaries and getting degraded. From the NTS, projections may also reach the hypothalamus, where activation (c-fos) has been observed in several nuclei including the paraventricular nucleus [65]. The powerful inhibitory actions of GLP-1 on the motor and secretory activity of the organs of the upper gastrointestinal tract are undoubtedly mainly transmitted via this pathway, since these activities are completely dependent on intact vagal nerve [66]. It is also possible that circulating GLP-1 may penetrate into the brain via the leaks in the blood brain barrier; indeed, the GLP-1 receptor is expressed in the regions where these leaks are found, including the area postrema, the median eminence, the arcuate nucleus and the subfornical organ [67,68]. Most likely, the therapeutic GLP-1 receptor agonists use this pathway to exert their actions on appetite and food intake. For GIP, similar mechanisms of action are not known to exist, but GIP may also access the brain via these leaks, and since there is expression of the GIP receptor in the brain [32], it cannot be excluded that some of the actions of GIP also involve the central nervous system.

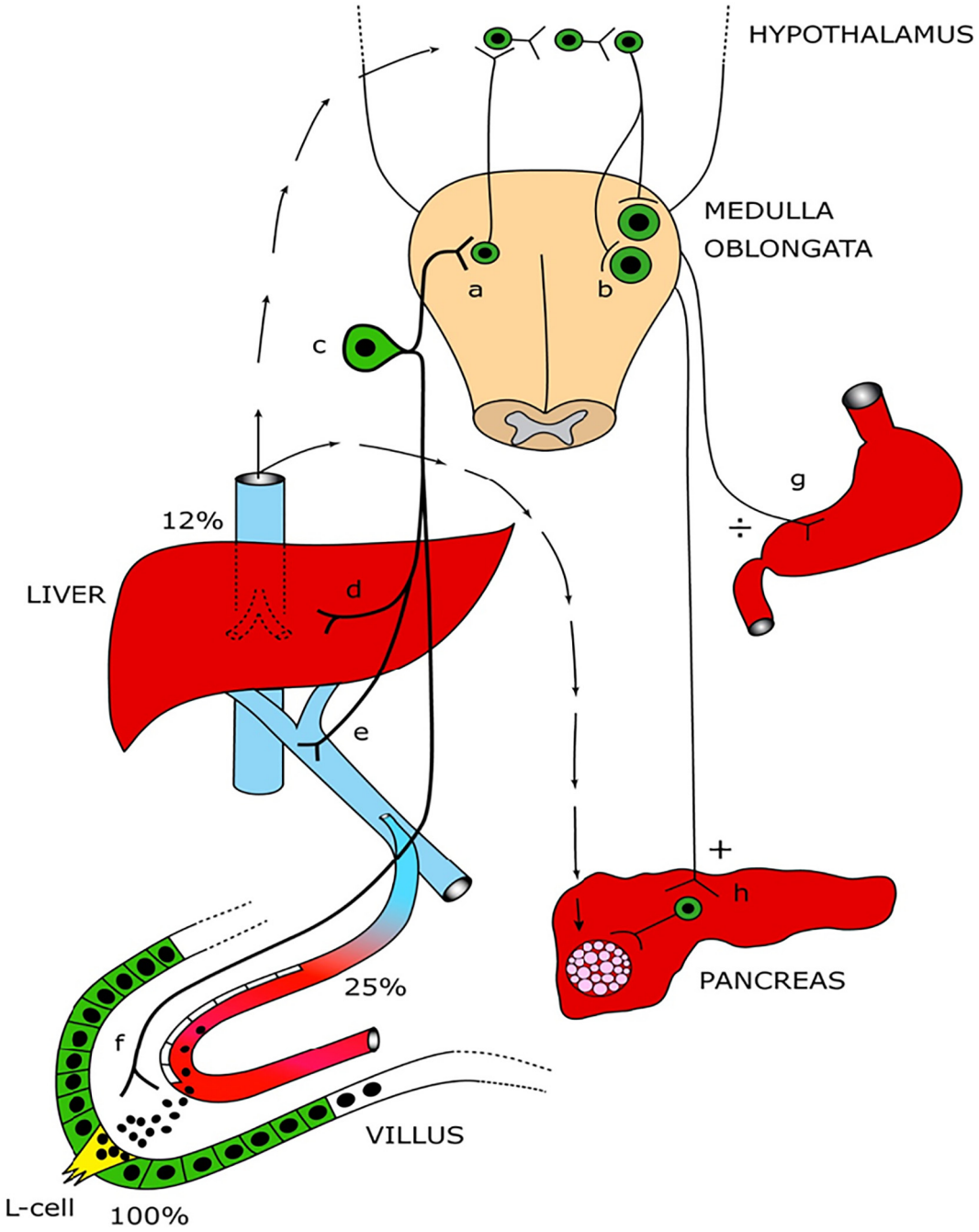

Fig. 4. Activation of long vago-vagal reflexes by GLP-1 before it gets degraded by dDPP-4 in the intestinal capillaries and the portal circulation. Schematic drawing of a villus with an L-cell secreting GLP-1 in the intact form (black dots) which may interact with GLP-1 receptors on sensory vagal afferents in the intestine (f), the portal vein (e), or the liver (d), originating in the nodose ganglion (c) and projecting to the nucleus of the solitary tract (a) in the brain stem. Here activated neurons may signal to the dorsal vagal motor complex (b) or to the hypothalamus. Activated efferent vagal neurons may signal to the stomach (g) and the pancreas (h). The percentages of GLP-1 surviving in the intact form are indicated. From Holst & Deacon [94] with permission.

3.5. The Physiological Roles of GIP and GLP-1: The Receptor Antagonists

What is the contribution of the two hormones to the overall incretin effect and which hormone is more important? Originally it was thought, based on infusion studies, that both hormones required elevated glucose levels to be effective, and their relative efficacy was debated on the light of this. However, in careful studies [45], in which the hormones were infused to reach precisely the concentrations observed in healthy individuals during meal intake and involving clamping of glucose levels at the fasting level and at levels corresponding to normal postprandial levels (up to 7 mmol/L), it was shown that both hormones acted on insulin secretion already at fasting glucose concentrations, and more so as glucose levels were increased, and that the two hormones stimulated insulin secretion about equally, GLP-1 a little bit more at the higher glucose concentrations. An important difference also emerged: one, GLP-1, strongly inhibited glucagon secretion, beyond the suppression caused by the glucose infusions, whereas the other, GIP, if anything, stimulated glucagon secretion [45]. But these experiments do not really tell us about the overall importance of the two hormones, and do not allow us to distinguish between their contributions, although previous studies of the exogenous hormones indicated that they would have additive effects [69].

For this, receptor antagonists are needed. For GLP-1, a receptor antagonist has been known since Raufman and Eng identified exendin-4 as a full agonist of the GLP-1 receptor and found that the truncated form, exendin 9–39, is a potent antagonist of the receptor [70]. The first experiments in humans with the new receptor antagonist were carried out a few years later [71,72]. The antagonist clearly led to increased glucose levels, but in those experiments the most conspicuous effect was increased levels of glucagon, confirming the role of GLP-1 in the control of glucagon secretion, although the effect in the fasting state was a surprise, since it would imply an action on pancreatic glucagon secretion of the basal, fasting levels of GLP-1, which are very low. Further experiments carried out with the antagonists confirmed the actions on glucagon secretion, but in experiments involving duodenal infusions of glucose, whereby the powerful actions of GLP-1 on gastric emptying rates were circumvented, it was clear that GLP-1 also, as predicted, plays a role for post glucose insulin secretion [73,74]. Indeed, in individuals with exaggerated GLP-1 secretion e.g. after gastric bypass operations, exendin 9–39 eliminated all the benefits of the operation on insulin secretion [75]. However, a paradoxical *increase* in insulin secretion has been seen in some individuals in most studies [71,72,76,77]. There is currently no clear explanation for this. It has been speculated that the elevated glucose concentrations resulting from antagonist infusion (a consequence of the elevated glucagon concentrations) may explain the increase [71]; indeed, the increases in glucagon are considerable and might contribute – after all, glucagon is a rather potent stimulant of insulin secretion. The effect on insulin secretion could be due to both systemic effects of the arterial hyperglucagonemia or local paracrine effects of the high concentrations of glucagon in the islets, which might stimulate the beta cells directly (via their glucagon receptors) [78]. In animal experiments, it was observed that blockade of one of the incretin receptors might be associated with increases in the secretion of the other [79]. This has not been confirmed in human studies.

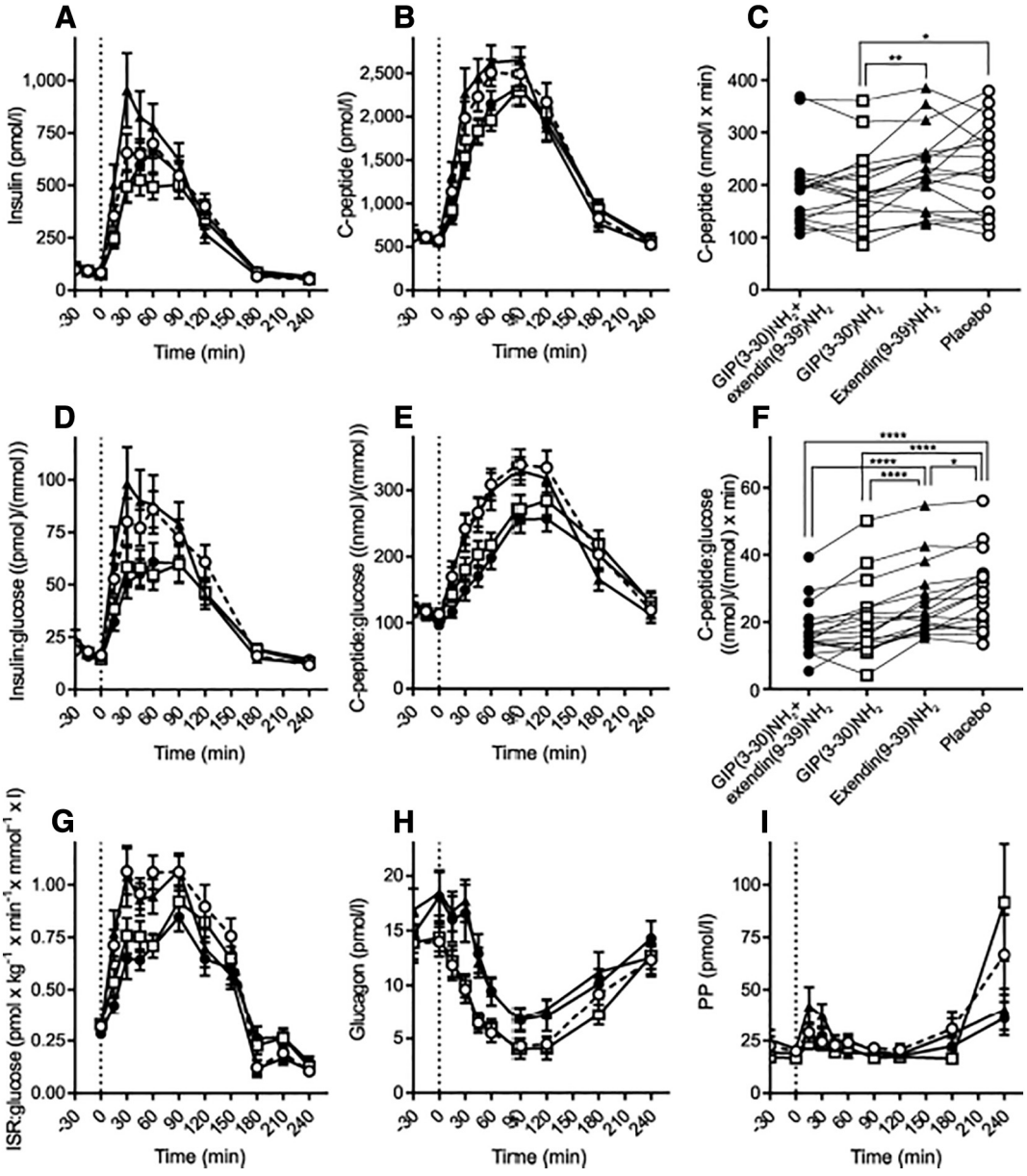

Fig. 5. Insulin, C-peptide and glucose responses from experiments in 17 healthy but overweight/obese subjects given an oral glucose tolerance test (75 g) at time 0 on 4 occasions: after placebo infusion; during infusion of the GLP-1 receptor antagonist exendin [9–38]; during infusion of the GIP receptor antagonist GIP (3−30)NH2; or during combined infusion of the two antagonists. Panel C shows areas under the curves for C-peptide; Panel E: ratios between C-peptide and glucose and panel F: AUC for these ratios. Asterisk in panels C and F indicate significant differences. Mean +/− SEM. From Gasberg et al. [86].

For GIP, a receptor antagonist suitable for human use was recently identified after systematic studies of various truncations of the GIP molecule in the laboratory of Mette Rosenkilde at the University of Copenhagen, which confirmed some receptor antagonistic properties of the truncated forms of GIP, GIP 3–42 and also GIP 5–42 [80,81]. The GIP molecule has a cleavage site for proteolytic endopeptidases (i.e. a pair of basic amino acid residues) at positions 32 and 33, and an early search for a truncated form of GIP corresponding to GIP 1–31 or 1-30amide (assuming that the carboxyterminal Gly would function as substrate for the amidating enzyme and donate the amino group to the preceding residue no 30) revealed presence of GIP 1-30amide in the alpha cells of the murine pancreas [82]. It was not possible to confirm this observation with sensitive assays for the GIP 1-30amide, but small amounts were indeed found in extracts of the gut and low concentrations were found in the circulation (but not after pancreatectomy) [83]. Given the sensitivity of this molecule towards DPP-4, it was assumed that the truncated form GIP 3–30NH2 would also exist. This form turned out to be a high potency antagonist on the human GIP receptor [84]. Being a natural product, it was also considered safe to administer this molecule to humans. It soon turned out that with a surplus of the antagonist, it was possible to block in humans most of the effect of "normal" doses of GIP on insulin secretion [84] and also to block its actions on adipose tissue blood flow and triglyceride uptake [85]. The results of antagonism of endogenous GIP are now also being investigated during mixed meal as well as oral glucose administration. In these studies [86], the GLP-1 antagonist, exendin 9–39, has also been given as well as a combination of GIP 3–30NH2 and Exendin-9-39.

Some representative results are shown in Fig. 5. It is seen, as expected, that both antagonists led to elevations of the plasma glucose concentrations in response to the oral glucose administration, and that the combination resulted in what appears to be an additive effect on blood glucose. Thus, it could be confirmed that both hormones act to lower postprandial glucose after a glucose challenge. Looking at insulin responses, a clear reducing effect was elicited by the GIP antagonist, whereas exendin 9–39 again inhibited insulin secretion in some individuals, but increased secretion in others (6 out of 18). Regarding glucagon, exendin 9–39 caused a marked increase in concentrations, whereas the GIP antagonist, if anything, lowered responses. The combination resulted in a near neutral response. Thus, it is clear that the effects of the two hormones on glucagon secretion are opposing. It is also clear that GIP appears more powerful as an incretin hormone than GLP-1, the actions of which may be concealed by its strong effect on glucagon secretion. The combination studies also lend some support to the theory that the lesser anti-incretin effectiveness of exendin 9–39 as compared to GIP 3–30NH2 may be related to the effects on glucagon secretion (as opposed to the theory holding increasing glucose concentrations responsible), since GIP 3–30NH2 has the opposite effects on glucagon secretion, but similar or even greater effects on plasma glucose. An estimate of the absolute incretin effect of the two hormones is difficult to extract from these experiments, since there were no intravenous infusions. The differences in insulin secretion between placebo and double antagonist experiments seem less than those obtained in experiments with isoglycemic intravenous and oral glucose challenges, but this comparison is invalid, since glucose concentrations are radically changed by the antagonists. The precise estimate therefore must await intravenous control studies. In addition, although it was shown that the doses of both antagonists resulted in major reductions of the effects of the exogenous peptides, there is no guarantee that the block of the endogenous hormones in the combination studies was complete.

Exendin 9–39 has been a great tool for the investigation of the numerous potential effects of endogenous GLP-1 [87], and apart from proving the role of GIP as an incretin hormone, it is expected that the new antagonist will provide a valuable tool for the elucidation of the many potential extrapancreatic effects of GIP [88]. In addition, the remarkable effects of the antagonist may point to a therapeutic potential [88].

4. Clinical Importance

As briefly alluded to above, loss of the incretin effect is one of the fundamental characteristics of T2DM [89]. The loss is thought to particularly influence and augment postprandial plasma glucose levels, and it is due to lack of insulinotropic effect of physiological levels of both GIP and GLP-1, as clearly demonstrated in infusion studies with physiological doses which in healthy individuals would dramatically increase insulin secretion [90]. Whereas GIP remains inactive regardless of dose, slightly supraphysiological doses of GLP-1 may stimulate insulin secretion to levels similar to those observed in healthy individuals in response to glucose alone [46,91]. One could say that supraphysiological levels of GLP-1 are able to restore the beta cell's responsiveness to glucose. Why is the beta cell response to the two hormones impaired in T2DM? This is not known, and it is not known why the two hormones differ in this respect, but it seems reasonable to assume that the underlying mechanism is related to and perhaps identical to that responsible for the cells' lack of response to glucose (the reason for which is also unknown!). The deficiency develops very early in course of T2DM [19] and a similar deficiency occurs during the development of insulin resistance [92,93]. An improvement may be observed after beta cell rest, as for instance after optimized glucose control [90]. The ability of larger doses of GLP-1 to restore beta cell glucose sensitivity is the background for the development of the GLP-1 receptor agonists for diabetes therapy.

5. Conclusion

Using isoglycemic oral and intravenous glucose challenges it can be demonstrated that up to 3 times more insulin is released during the oral administration. This is due to the effects of the incretin hormones, GLP-1 and GIP, and from studies with receptor antagonists for both hormones we now know that lack of both hormones deteriorate glucose tolerance, apparently with GIP being quantitatively the most important one in healthy individuals. This contrasts greatly with findings in patients with T2DM, in whom the GIP effects is largely lost, whereas supraphysiological doses of GLP-1 may restore the defective beta cell response to glucose. The marked glucose-lowering effects of newly developed GLP-1 RAs support that restoration of the incretin effect in type 2 DM is one of the most powerful therapeutic approaches to type two diabetes.

Conflict-of-Interest

JJH has collaborated with several different pharmaceutical companies during the last 30 years; is currently receiving speaker honoraria from NovoNordisk and MSD and is on advisory boards for NovoNordisk. The author is currently supported by an independent grant from the NovoNordisk Foundation to the NNF Center for Basic Metabolic Research.

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